**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| In re application of: | |
| NAKAI *et al.* | Art Unit: 1633 |
| Application No.: 09/897,988 | Examiner: Maria Marvich |
| Filing Date: July 5, 2001 | Attorney Ref. No.: US-142O |
| For: METHOD FOR PRODUCING SUBSTANCE UTILIZING MICROORGANISM | Confirmation No.: 1677 |

**BRIEF FOR APPELLANT**

**Mail Stop Appeal Brief - Patents**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

COMES NOW the Appellant to present this Brief in support of the appeal of the final rejections of Claims 1, 6, and 12-17 in the above-captioned patent application. The Notice of Appeal having been timely filed on November 5, 2010, this Brief is due to be filed on January 5, 2011.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. If, however, additional extensions of time are necessary to prevent abandonment of this application or dismissal of this appeal, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and the Commissioner is hereby authorized to charge fees necessitated by this paper, and to credit all refunds and overpayments, to the credit card authorized on the attached PTO-2038.

For the following reasons, Appellant respectfully submits that the final rejection of each of Claims 1, 6, and 12-17 in this application is in error, and therefore respectfully requests reversal of the rejections.

## TABLE OF CONTENTS

## I. Real Party in Interest

The real party in interest is Ajinomoto Co., Inc, a corporation of Japan.

## II. Related Appeals and Interferences

Rejections of claims in this application were previously appealed to the Board of Patent Appeals and Interferences, Appeal No. 2009-4917, which were reversed in a Decision dated August 11, 2009. A copy of the Decision is attached at Appendix C. 37 C.F.R. § 41.37(c)(1)(x).

## III. Status of Claims

Claims 1, 6 and 12-17 are pending. Claims 1, 6 and 12-17 stand finally rejected in the Office Action dated June 11, 2010, as confirmed in the Advisory Action dated October 13, 2010, and are the subject of this appeal.

## IV. Status of Amendments

All amendments to the claims have been entered, including the Amendment After Final filed on September 13, 2010.

## V. Summary of Claimed Subject Matter

**Claim 1**: A method for producing an L-amino acid comprises culturing a bacterium belonging to the genus *Escherichia* or a coryneform bacterium in a medium, and collecting said L-amino acid from said medium. The bacterium has an ability to produce and accumulate the L-amino acid in the medium and has been modified so to have enhanced activity of cytochrome bo-type oxidase by a method selected from the group consisting of i) increasing the copy number of a gene coding for said oxidase, ii) modifying an expression regulatory sequence of said gene, and iii) combinations thereof. [page 8, line 25 through page 18, line 25]

## VI. Ground of Rejection to Be Reviewed on Appeal

The Office Action includes a single (compound) rejection to be reviewed on appeal, the

rejection of Claims 1, 6, and 12-17 under 35 U.S.C. § 103(a) as reciting subject matters that allegedly would have been obvious, and therefore allegedly are unpatentable, over U.S. Patent No. 5,830,716, granted to Kojima *et al*, in view of Calhoun *et al* (J. Bacteriol. 1993 May; 175(10), pp. 3020-3025), or Ciccognani *et al* (FEMS Microbiology Letters 94, 1992, pp. 1-6), or Kusumoto *et al* (Arch. Microbiol., 200, Vol. 173, pp 390-397), or Sone *et al* (Collection of Summaries of Lectures made at the Meeting of Japan Bioengineering Association, Sept. 15, 1995, p. 10).

## VII. Argument

*A. Introduction*

This application describes and claims methods of producing amino acids using a microorganism. Many organisms acquire energy required for survival by respiration. In the respiration of microorganisms, the function of various enzyme complexes is generally dependent on the species or the growth environment, and energy acquisition efficiency can vary significantly. Carbohydrates, proteins and aliphatic acids are converted into acetyl-CoA by glycolysis, β-oxidation, and so forth, and decomposed in the citric acid cycle. Then, the energy preserved in the form of NADH is used for proton excretion from microbial cells with the aid of NADH dehydrogenase (NDH), and an electron transfer system consisting of oxidoreductases, and thereby a proton concentration gradient, is formed between the inside and outside of the cytoplasmic membrane. This proton concentration gradient is the driving force of adenosine triphosphate (ATP) synthesis. At this time, pathways of electron transfer include pathways showing high and low proton excretion ability, depending on the combination of NDH and oxidoreductases. It is thought that a pathway of high proton excretion ability shows high energy efficiency and a pathway of low proton excretion ability shows low energy efficiency. Thus, one kind of microorganism can simultaneously contain a plurality of respiratory chain electron transfer pathways in parallel, and those pathways can include those of high energy efficiency and low energy efficiency.

Two types of NDHs and terminal oxidases exist in the respiratory chain of *Escherichia coli* under aerobic conditions. That is, NDH-1, encoded by the *nuo* operon, is known to have high

energy efficiency, and NDH-II, encoded by *ndh*, is known to have low energy efficiency. Furthermore, cytochrome bo-type oxidase, encoded by the *cyoABCD* operon, and classified as a SoxM type is known to be highly energy efficient, and cytochrome bd-type oxidase, encoded by *cydAB*, is known to be poorly energy efficient. Although it is known that the levels of expression of these respiratory chain enzymes vary in response to their growth environment, much is unknown about the physiological meaning of their expression patterns.

Furthermore, *Corynebacterium glutamicum* contains a cytochrome bc1 complex and at least two kinds of terminal oxidases, SoxM type oxidase and cytochrome bd type oxidase. This shows that there are two kinds of electron transfer pathways from a quinone pool to an oxygen molecule and include a pathway utilizing cytochrome bc1 complex and SoxM type oxidase, and a pathway utilizing only the cytochrome bd type oxidase. It is thought that the former is an electron transfer pathway of high energy efficiency in which the proton transfer value for transfer of one electron is high, and the latter is an electron transfer pathway of low energy efficiency in which proton transfer value for transfer of one electron is low.

As for the terminal oxidase of *E. coli*, in a comparison of growth yields in aerobic cultures of a mutant strain having only the cytochrome bo-type oxidase, a mutant strain having only the cytochrome bd-type oxidase, and a wild-type strain having both, the growth yield will be the lowest in the mutant strain having only the cytochrome bd-type oxidase, and it depends on the kind and energy acquisition efficiency of terminal oxidase.

Furthermore, the energy efficiency of mutants of deficient in some respiratory chain enzymes has been reported. However, there have been no reports concerning a change in energy efficiency by amplification of a respiratory chain gene providing high efficiency such as those for NDH-I and SoxM type oxidase, and an attempt to utilize such for production of substances such as amino acids has also not been reported. Furthermore, no attempts have been made to delete a respiratory chain enzyme of low efficiency such as NDH-II and cytochrome bd-type oxidase for production of substances.

Energy is required for biosynthesis of substances such as L-amino acids and nucleic acids in living bodies. Most energy used consists of the reducing powers of NADH, NADPH, and so

forth, and energy preserved as ATP. Therefore, if the energy supply utilized in the production of target substances was increased in methods for producing target substances utilizing microorganisms, production of the target substances can be improved. Based on such a concept, one of numerous aspects of the present invention includes methods for producing a target substance.

The inventors of the present invention conceived that a microorganism having an increased energy supply could be constructed by enhancing a respiratory chain pathway having high energy acquisition efficiency or making deficient a respiratory chain pathway with low energy acquisition efficiency. Specifically in *E. coli*, strains considered to have improved energy efficiency were prepared by amplifying a gene coding for cytochrome bo-type oxidase, a respiratory chain enzyme of high energy efficiency, or deleting a gene coding for NDH-II, a respiratory chain enzyme of low energy efficiency. Then, L-amino acid production was performed using these strains and it was found that the L-amino acid production was improved in strains whose energy efficiency was improved.

To simplify consideration of the rejections of the various claims, the prior art documents relied upon in the Office Action will be discussed after a brief review of the law of claim construction and obviousness under section 103.

*B. Legal Standard*

**Claim Construction**

Claim construction begins with the words of the claims. *Karlin Tech., Inc. v. Surgical Dynamics, Inc.*, 177 F.3d 968, 971 (Fed. Cir. 1999). Claim language should be interpreted as one reasonably skilled in the art would have interpreted the claim at the time of the patent application date. *Vivid Techs., Inc. v. American Science & Engineering, Inc.*, 200 F.3d 795, 804 (Fed. Cir. 1999); *Wiener v. NEC Elec., Inc.,* 102 F.3d 534, 539 (Fed. Cir. 1996). Where the claim term has no specialized meaning to persons of skill in the art, the ordinary meaning of the words to those of ordinary skill in the art controls, unless the evidence indicates that the inventor used them differently. *Karlin*, 177 F.3d at 971. Such evidence includes the specification and prosecution

history, both of which must be analyzed to determine if the inventor limited or redefined any of those terms. *Watts v. XL Sys., Inc.*, 232 F.3d 877, 882-84 (Fed. Cir. 2000); *Vivid Techs.*, 200 F.3d at 804. If claim language is not clear on its face, then intrinsic evidence also should be consulted to resolve the lack of clarity. *Interactive Gift Express, Inc. v. Compuserve, Inc.*, 256 F.3d 1323, 1331 (Fed. Cir. 2001).

The standard for the interpretation of a claim and its terms during prosecution of an application for patent in the U.S. Patent and Trademark Office ("PTO") is that the PTO must give claim terms their "broadest reasonable interpretation consistent with the specification." *In re Suitco Surface, Inc.*, No. 2009-1418, slip. op. at 6 (Fed. Cir., April 14, 2010). Thus, an interpretation of a claim term by the PTO during prosecution of a patent application which is not consistent with the meaning of that term as used in the application's specification is not permitted under the current jurisprudence.

**Obviousness under 35 U.S.C. § 103**

A patent claim is invalid for obviousness if the differences between the claimed subject matter and the prior art are such that the claimed subjected matter as a whole would have been obvious at the time of the invention to a person of ordinary skill in the relevant art. 35 U.S.C. § 103(a). The determination of obviousness is a legal conclusion based on underlying factual considerations. *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1, 17 (1966). These factual inquiries include: the scope and content of the prior art; the differences between the prior art and claims at issue; the level of ordinary skill in the pertinent art; and objective evidence of nonobviousness (*i.e.*, secondary considerations). *Graham*, 383 U.S. at 17; *KSR International Co. v. Teleflex Inc. et al.*, 550 U.S. ___, No. 04-1350, slip op. at 2 (April 30, 2007); *Brown & Williamson Tobacco Corp. v. Phillip Morris Inc.*, 229 F.3d 1120, 1124 (Fed. Cir. 2000); *DyStar Textilfarben GmbH & Co. Deutschland KG v. C. H. Patrick Co.*, 464 F.3d 1356 (Fed. Cir., 2006).

It is against this factual background that the ultimate determination of obviousness is made, *i.e.*, the claimed invention is obvious if the differences between it and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person

of ordinary skill in the art. 35 U.S.C. § 103(a). "In line with th[e] statutory standard [of 35 U.S.C. §103], [the] case law provides '[t]hat consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have a reasonable likelihood of success, viewed in light of the prior art.' Two requirements are contained in this criterion." *Brown & Williamson Tobacco Corp.*, 229 F.3d at 1124 (*quoting In re Dow Chem.*, 837 F.2d 469, 473 (Fed. Cir. 1988)).

The U.S. Supreme Court recently addressed the obviousness of a combination of known elements. Although a rigid application of the Court of Appeals for the Federal Circuit's "teaching, suggestion, or motivation" test was rejected, the Court stated that "a combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." *KSR, slip op.* at 12. For example, the Court explained, when the prior art elements work together in an unexpected and fruitful manner, a finding of non-obviousness is supported. *Id.* (*citing United States v. Adams,* 383 U.S. 39, 40 (1966)). If, however, the combination of old elements does no more than they would in separate, sequential operation, even though the combination might perform a useful function, the combination is likely obvious. *Id.* at 13 (*citing Anderson's-Black Rock, Inc. v. Pavement Salvage Co.,* 396 U.S. 57 (1969)). Finally, the Court stated that "[i]f a person of ordinary skill can implement a predictable variation, §103 likely bars its patentability." *Id.* (*citing Sakraida v. AG Pro, Inc.,* 425 U.S. 273 (1976)). Nevertheless, the Court in *KSR* still required that there be a reason or purpose for modifying the prior art to arrive at the claimed invention, in order to find the claimed subject matter unpatentable under section 103(a). *Id.* at 14-15 ("Although common sense directs one to look with care at a patent application that claims as innovation the combination of two known devices according to their established functions, it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does.").

Thus, while the Supreme Court in *KSR* ruled that the requirement, in the jurisprudence of the Court of Appeals for the Federal Circuit, for a "teaching, suggestion, or motivation" ("TSM" test) to make up for the deficiencies in the prior art to meet the claimed invention, cannot be rigidly applied, the Federal Circuit had already articulated that its test was flexible. *See*, *e.g., DyStar*

*Textilfarben*, 464 F.3d at 1367 ("Our suggestion test is in actuality quite flexible and not only permits, but *requires*, consideration of common knowledge and common sense") (emphasis in original); *Alza Corp.* v. *Mylan Labs., Inc.*, 464 F.3d 1286, 1291 (Fed. Cir. 2006) ("There is flexibility in our obviousness jurisprudence because a motivation may be found *implicitly* in the prior art. We do not have a rigid test that requires an actual teaching to combine . . ."." ) (emphasis in original). It is therefore plain that *KSR* did not reject the TSM test, but only its application to the facts before the Court in that case, and it is thus still a requirement for a rejection under section 103 that there be some rational reason articulated by the PTO why a person of ordinary skill in the art would modify the prior art to arrive at the claimed invention.

The mere existence in the prior art of all of the individual features recited in a claim directed to a combination, is not sufficient evidence to preclude patentability under section 103(a). In a case recently decided by the Federal Circuit, *Dupuy Spine, Inc. v. Medtronic Sofamor Danek, Inc.*, No. 2008-1240, -1253, 1401 (June 1, 2009), the Court found that the hypothetical combination of simple mechanical features in a pedicle screw would not have been obvious to a person of ordinary skill in the art. Despite the fact that the individual features recited in the claim at issue were collectively present in two prior art documents, the Court found that a person of ordinary skill in the art, upon a full reading of the documents, would not have found the combination to have been obvious. Addressing the "predictability" portion of *KSR*, the Court stated:

> Although predictability is a touchstone of obviousness, the "predictable result" discussed in KSR refers not only to the expectation that prior art elements are capable of being physically combined, but also that the combination would have worked for its intended purpose. . . . As the Supreme Court explained, "The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." . . . . The Supreme Court went on to state that "when a patent 'simply arranges old elements with each performing the same function it had been known to perform' and yields no more than one would expect from such an arrangement, the combination is obvious." . . . The opposite conclusion would follow, however, if the prior art indicated that the invention would not have worked for its intended purpose or otherwise taught away from the invention. . . . An inference of nonobviousness is especially strong where the prior art's teachings undermine the very reason being proffered

as to why a person of ordinary skill would have combined the known elements.

(emphasis in original; internal citations omitted) *Dupuy*, slip op. at 13-14. Thus, in assessing a hypothetical combination of features present in the prior art, the obviousness inquiry does not stop at a mere identification of the elements, but must also take into consideration the functions of the elements, their intended purposes, and if the prior art teaches away from the claimed combination.

*C. The Prior Art*

The Office Action cites five prior art documents in an attempt to formulate a *prima facie* case of obviousness.

(i). *Kojima*

*Kojima* is the primary reference in each of the alternative rejections. This reference describes methods of producing target substances, such as amino acids using bacterial fermentation. Kojima also discusses increasing the bacteria's ability to produce NADPH from NADH by increasing the expression of nicotinamide nucleotide transhydrogenase. Kojima also teaches that *E.coli* and Coryneform bacteria are well-known for producing amino acids via fermentation. Kojima is specifically cited by the Examiner for teaching methods of using bacteria for production of amino acids, and that *E. coli* and *Coryneform* bacteria are well-known for use in producing threonine, lysine, and phenylalanine. It is acknowledged by the Examiner that Kojima does not include any discussion of high energy efficiency pathways and low efficiency pathways, or the effects of altering these pathways (see First Office Action dated December 3, 2009, page 3).

The Office Action cites to 4 secondary references as curing this deficiency, each in the alternative, and so each combination of Kojima with each of the secondary references will be addressed in turn.

(ii). *Calhoun*

*Calhoun* describes a study of the energy efficiency of *E. coli*, and specifically the effects of mutations in components of the aerobic respiratory chain, including NDH-1, NDH-2, bd-type

oxidase, and bo-type oxidase. As each of these components are determined to be important to the bioenergetic efficiency of the respiratory chain, Calhoun 'genetically eliminates' each of these components to study the effect on specific rates of oxygen consumption. They conclude that bd-type oxidase is less efficient than bo-type oxidase, both NDH-1 and NDH-2 are utilized to a significant degree, and electron flux is directed through the bo-type oxidase. Significantly, there is no discussion of producing amino acids, nor the effect of any of the studied mutations on possible amino acid production, nor the effect of the change in oxygen consumption rates on the production of amino acids.

(iii). *Ciccognani*

*Ciccognani* describes the effects of copper deficiency in a culture of *E.coli* on the carbon monoxide binding to the cytochrome bo oxidase. To study this effect, the bo-oxidase level in *E.coli* was increased while the bd oxidase levels were decreased. The effects of limiting copper on the components of the cytochrome o complex were studied, and it was found that the concentration of cytochrome o was not significantly altered. Furthermore, growth yields in the cultures were unaffected when using a copper-limited medium. Finally, the CO-binding properties of the oxidase were found to be decreased under copper-depleted conditions. Again, there is no discussion of producing amino acids, nor the effect of any of the studied mutations on possible amino acid production.

(iv). *Kusumoto*

*Kusumoto* describes the activity and primary structure of cytochrome bd from *Corynebacterium glutamicum.* The activity was found to be menaquinol oxidase, but was low and could be significantly increased by pre-incubating with menaquinones. The genes of the subunits were cloned and the deduced amino acid sequence characterized. Again, there is no discussion of producing amino acids, nor the effect of any of the studied mutations on possible amino acid production.

(v). *Sone*

*Sone* describes the growth yield in wild-type *E.coli,* containing both a bo- and bd-type oxidase, a strain lacking the bd-type, and a strain lacking the bo-type. It was found that the highest growth yield was with the strain with the bo-type only, followed by the wild-type strain, and the lowest was the strain with only the bd-type. Sone concluded that the growth yield depends on the kinds of terminal oxidases and the energy yield of each.

C. *The rejection of Claims 1, 6, and 12-17 under 35 U.S.C. § 103(a) is in error*

The Office Action makes rejections, cited in the alternative, combining a single primary reference, Kojima, with four separate secondary references, each standing alone with Kojima. The first rejection is, therefore, the rejection of claims 1, 6, and 12-17 over Kojima in view of Calhoun. As stated *supra,* Kojima is cited for allegedly teaching that *E.coli* and Coryneform bacteria are well known in production methods of threonine, lysine, and phenylalanine wherein the cells are engineered to improve production by altering a biochemical cellular pathway (see Final Rejection of June 11, 2010, page 2). It is acknowledged that Kojima's description of altered biochemical pathways does not include one wherein the high energy efficiency pathways, such as cytochrome bo, or low efficiency pathways, such as cytochrome bd, are altered by elimination.

The secondary reference, Calhoun, is cited for allegedly teaching, both explicitly and inherently, strains in which the bo enzyme is increased and NDH-II is decreased, and the effects on cell growth of these modifications. However, it should be noted that Calhoun only disclose strains with deletion of respiratory chain enzymes (see Table 1 at page 3021), but Calhoun never discloses a strain with enhanced cytochrome bo-type oxidase activity. Furthermore, the focus of Calhoun is clearly on the bioenergetic efficiency of the respiratory chain when the components of these pathways are altered, and no mention is made on the effect of any of these changes on amino production by the cell. The Office Action states "Calhoun explicitly teaches that to increase growth efficiency, one would eliminate NDH-II or bd and increase bo." This conclusion is somewhat overreaching, based on Calhoun, as explained below. Furthermore, it is not logical to

conclude that, even if *arguendo*, elimination of NDH-II and increasing bo oxidase leads to increased cell growth that production of L-amino acids is an obvious or expected result. Calhoun only discusses the effect of these various changes, such as a decrease in NDH-II in the cell, on the cell growth, and does not mention or imply that there is any effect, either positive or negative, on L-amino acid production by the cell. In fact, Calhoun states "[t]he goal of this work is to determine the consequences of specific respiratory defects on the ***growth*** of *E. coli*" (emphasis added). However, when the bacterial growth yield is high, new construction of cellular components, such as cell walls, becomes necessary, and thus more carbon is consumed in cellular component synthesis. Therefore, one of ordinary skill in the art would know that increases in cell growth does not correlate to, or even imply, an improvement in L-amino acid production. To the contrary, L-amino acids are preferably produced by fermentation under conditions when the ***cell growth is suppressed*** in order to prevent such consumption of carbon sources for the formation of the cell components. Therefore, the yield of the target substances such as L-amino acids is decreased when the cell growth is increased. The person of ordinary skill in the art would know from reading Calhoun that making the changes taught by Calhoun, such as decreasing NDH-II activity, will result in increased cell growth, but the person of ordinary skill in the art would also know that an increase in cell growth does not mean an increase L-amino acid production. In fact, the person of ordinary skill in the art would know that the L-amino acid production will actually be decreased as cell growth increases; and therefore, there is no logical reason or motivation to combine the teachings of Calhoun with those of Kojima and arrive at the claimed invention.

Ciccognani also cannot be combined with Kojima to arrive at the claimed invention. Ciccognani is cited for allegedly teaching methods of culturing E. coli in which an enzyme of the high-energy efficiency pathway is enhanced and an enzyme of the low-energy efficiency was deficient. However, Ciccognani is actually a study of the binding properties of oxidases in *E. coli* and the effect of the depletion of copper on cell growth. It is shown that copper deficiency during growth results in depletion of copper from the oxidase. There is no discussion or suggestion of improved L-amino acid production. Therefore, similar to the combination of Calhoun and Kojima, there is no logical reason to combine Ciccognani and Kojima. This is because, as pointed

out *supra,* there is no direct relationship between cell growth and L-amino acid production, and studies showing effects on cell growth under certain conditions such as that by Ciccognani bear no relationship to L-amino acid production. One of ordinary skill in the art would know that changes in cell growth does not correlate to, or even imply, an improvement in L-amino acid production. As Ciccognani does not teach, suggest, or even imply that any of the manipulations taught in their study relate in any way to L-amino acid production, and the person of ordinary skill in the art would know that changes in cell growth does not indicate an increase in L-amino acid production, there is no logical reason to combine the teachings of Ciccognani with those of Kojima.

Kusumoto also cannot be combined with Kojima to arrive at the claimed invention. Kusumoto is cited for allegedly teaching cells which are used in a method for producing amino acid, and that such cells can be altered to obtain improved amino acid production by altering the aerobic metabolism of the cell, specifically by deleting the low efficiency gene. The Office Action (see first Office Action of December 4, 2009, page 3) goes on to allege that Kusumoto directly link amino acid production with the growth yield and energy efficiency of the cell. Although Kusumoto does teach a cytochrome ***bd type*** oxidase gene; Kusumoto does not teach or suggest modifying a cell to enhance the activity of cytochrome ***bo-type*** oxidase nor the use of such a modified cell in the production of L-amino acids. Kusumoto states "in order to improve the growth of cells and synthesis of amino acids, it is important to understand the aerobic energy metabolism, more specifically the respiratory proton pumps in the bacterium" (page 390, right column, lines 4-7); however, one of ordinary skill in the art would not understand from this disclosure, either combined or not combined with Kojima, how to modify the respiratory proton pumps to achieve improved production of L-amino acids since this is a purely speculative statement. As Kusumoto does not teach, suggest, or even imply that enhancing activity of the ***bo enzyme*** may relate in any way to L-amino acid production, there is no logical reason to combine the teachings of Kusumoto with those of Kojima.

Finally, Sone also cannot be combined with Kojima to arrive at the claimed invention. Sone is not discussed in the Office Action of December 4, 2009 except for the brief statement that Sone teaches strains with bo cytochrome oxidase activity and deficient cytochrome bd oxidase

activity which have enhanced growth. However, Sone evaluated cytochrome bo-type oxidase and cytochrome bd-type oxidase, and reports that the growth of the strains with either one of these enzymes and a wild-type strain was on the order of the bo strain (cytochrome bo-type oxidase strain) > wild type strain > bd strain (cytochrome bd-type oxidase strain). However, this document only discloses the effect of cytochrome bo-type oxidase on oxygen consumption, proton transport and growth yield (growth rate), and does not disclose the effect of cytochrome bo-type oxidase on L-amino acid production. Similar to the above points, improvement of oxygen consumption, proton transport and growth does not correlate to improvement of L-amino acid production. That is, when the bacterial growth yield is high, new construction of cell components such as cell walls is necessary, and thus more carbon is consumed in the synthesis of cell components. Therefore, one of ordinary skill in the art would understand that carbon flux into L-amino acid synthesis pathway may be reduced if cytochrome bo-type oxidase activity is enhanced, and so there is no reason or motivation based on the disclosure of Sone to combine these teachings with those of Kojima, in that there is no suggestion or motivation provided to enhance cytochrome bo-type oxidase activity for the production of L-amino acid. Furthermore, L-amino acids are preferably produced by fermentation under conditions when the cell growth is suppressed, in order to prevent that carbon sources are consumed for the formation of the cell components and the yield of the target substances such as L-amino acid is decreased thereby.

In regards to the final two secondary references, Kusumoto and Sone, the Office Action cites to the Office Action from the corresponding Japanese Application: "it is suggested to use enzyme genes relating to the electron transfer system in the respiratory chain, in order to improve the growth of cells and to produce the useful substance, such as, amino acid, with the better energy efficiency." However, these sentences are the opinion of the Japanese Examiner, and neither Kusumoto nor Sone cited in the Japanese Office Action teach or suggest the use of enhanced cytochrome bo-type oxidase for producing L-amino acids. It should be noted that the corresponding Japanese application is now granted for patent after withdrawal of the rejection.

Therefore, there is no logical reason or motivation to combine any of the secondary references with Kojima, with the expectation to arrive at the claimed invention. None of the

secondary references relate their respective cellular manipulations regarding bo-type oxidase with any indication or hint that such a manipulation will increase, or even effect, production of L-amino acids by the cell. As there is no logical reason to combine these various teachings with the primary teaching of Kojima, the claimed invention cannot be obvious over these references.

As argued above, improved growth yield does not necessarily lead to an improvement in the production of L-amino acids, and the person of ordinary skill in the art would not be motivated to expect production of L-amino acids by making any cellular alteration that increases cellular growth. That is, when the bacterial growth yield is high, new construction of cell components such as cell walls is necessary, and thus more carbon is consumed in the synthesis of cell components, rather than in the biosynthesis of amino acids. One of ordinary skill in the art would understand that carbon flux into L-amino acid synthesis pathways is reduced when cytochrome bo-type oxidase activity is enhanced; and therefore, so one of ordinary skill in the art would never have been motivated to enhance cytochrome bo-type oxidase activity for the production of L-amino acids.

As support for this argument, Applicants submitted Exhibit A (Eggeling, et al., Appl. Microbiol. Biotechnol. 1998, 49(1):24-30) and Exhibit B (U.S. Patent No. 5,763,230, De Hollander) in response to the Office Action of June 11, 2010, which was considered and entered in the Advisory Action of October 13, 2010. Eggeling discloses that the rate of L-lysine excretion is improved by limiting growth (Tables 2 and 3), and includes the following description in the paragraph bridging from page 29 to page 30:

*"In conclusion, the present study shows that even a classically obtained, very good amino-acid-producing strain can be improved. However, the improvement does not only consist in the removal of a bottleneck, but is due to a subtle flux redistribution at the dehydrogenase/synthase branch point. Combined with this redistribution is an introduced growth limitation, which results in increased availabilities of metabolites within the central metabolism. In fact, for years process engineering has been using extracellular constraints, like limited supply of ammonium or of any medium components to restrict growth, thereby extending the period of increased product accumulation (Kiss and Stephanopoulos 1991; Konstantinov et al. 1991). This is suggested to be due to an increased availability of intracellular precursors. The present case of dapA demonstrates a relation of flux increase towards product with an intracellularly introduced growth limitation. Therefore, similar growth limitations, introduced by recombinant DNA techniques, are proposed as an attractive means for the improvement of further metabolite*

*production processes.*" (emphasis added).

De Hollander discloses that an L-amino acid can be produced efficiently by limiting growth, such as by limiting one or more necessary nutrients (see column 1, line 65 – column 2, line 4):

"*Different types of nutrient limitation can be employed. Carbon source limitation is most often used. Other examples are limitation by the nitrogen source, limitation by oxygen, limitation by a specific nutrient such as a vitamin or an amino acid (in case the microorganism is auxotrophic for such a compound), limitation by sulphur and limitation by phosphorous.*"

Column 2, lines 44-51 also can be cited in support:

"*According to the present process the steady state (or pseudo-steady state in fed-batch culture) is just phosphorous limited, with only a slight accumulation of residual sugar, or just in the region of carbon-phosphorous double limitation with both limiting nutrients practically exhausted. Advantageously the present invention resulted in an improved yield of product on consumed carbon source.*"

Finally, column 3, lines 50-55 are also representative:

"*Biomass production is an inevitable by-product of an amino acid fermentation. It is often difficult to find a suitable and economic outlet for this by-product. One of the advantages of the use of phosphorous limitation or phosphorous-carbon double limitation is a great reduction in biomass production.*"

Therefore, the state of the art supports the above arguments, in that one of ordinary skill in the art would never have been motivated to combine Kojima with Calhoun, Ciccognani, Kusumoto, or Sone and arrive at the claimed method of producing amino acids. Furthermore, even if there was a motivation or even a practical reason to combine Kojima with the other documents, it is quite doubtful from the disclosure of Kojima whether the growth yield could be improved by enhancing cytochrome bo-type oxidase activity in L-amino producing bacteria. That is, Kojima (U.S. Patent No. 5,830,716) discloses in column 3, lines 14-19:

"*As for L-amino acid biosynthesis pathways, intravital component which cannot be effectively utilized are often produced through the process of biosynthesis of desired L-amino acids from glucose. It is assumed that such components are ordinarily oxidized through the TCA cycle, resulting in generation of a large amount of NADH.*"

Since a large amount of NADH exists in cells of L-amino acid producing bacteria, one of

ordinary skill in the art would consider that a sufficient amount of ATP is already present in the cells of L-amino acid producing bacteria, regardless of energy efficiency of respiratory chain pathways and would never expect that growth yield of L-amino acid producing bacteria could be enhanced by modifying the activity of the respiratory chain pathway, much less L-amino acid productivity.

The Advisory Action, in addition to clarifying the Office Action as to which claims were rejected, provided several additional comments. More specifically, the Advisory Action argued that the teachings of Eggeling are not commensurate with the "instant teachings" (see Advisory Action, page 2). Although it is not clear why Eggeling must be commensurate with the 'instant teachings' [does the Examiner mean the teachings of the invention or the teachings of the cited prior art?], Eggeling is cited for a general priniciple that would influence one of ordinary skill in the art when evaluating the cited prior art, that is, the state of the art. Specifically, Eggeling shows that one of ordinary skill in the art would likely want to ***limit growth*** of a bacterial cell if the goal is to produce amino acids, regardless of what other manipulations are being conducted. Generally, each of the secondary references teaches some increase in cell growth, and the Office Actions repeatedly state that such increase in cell growth would logically lead the person of skill in the art to expect an increase in amino acid production by the cells. However, Eggeling shows that this is not the case, and in fact, one of ordinary skill it the art would actually expect the opposite.

Similarly, De Hollander teaches methods of improving L-lysine wherein phosphorus and/or carbon sources are limited, and that as a direct result biomass is reduced (see e.g. col 3, line). According to the Examiner, figure 1 demonstrates that as biomass increases so does the yield of L-lysine unless carbon and/or phosphorus is limited (figure 2 and 3). However, although the amount of L-lysine production is proportional to the amount of biomass at a P/C ratio of about 1.2 or less (P limitation condition), the amount of L-lysine production does not increase together with the increase in the amount of biomass at a PC ratio of about 1.2 to 4.2 (P and C double limitation condition). That is, it cannot always and consistently be said that as biomass increases so does the yield of L-lysine. The Advisory Action argues, similar to Eggeling, that De Hollander fails to provide adequate elucidation of recombinant engineering methods related to improved energy

efficiency and growth requirements. However, again, these references are provided as teachings of general principles and to show what one of ordinary skill in the art would have expected based on the cited references in light of the state of the art at the time of the invention. Clearly, one would not have necessarily expected that manipulations, regardless of their specifics, resulting in increased cellular growth (secondary reference teachings) would result in production of amino acids (claims). Such citations are not showing a 'known disadvantage in an old device', as cited by the Examiner in the Advisory Action, but are showing the state of the art and what one or ordinary skill in the art would have expected based on the cited teachings. Furthermore, the exhibits of Eggeling and De Hollander are not presenting 'evidence' or data to show the prior art teachings are not proper, which would necessitate such being commensurate in scope with the claimed invention, but are merely evidence showing the state of the art regarding the cited teachings and what one or ordinary skill in the art would have expected. Therefore, the citations provided in the Advisory Action are moot and irrelevant.

Therefore, there is no logical reason or motivation to combine any of the secondary references with Kojima, with the expectation to arrive at the claimed invention. None of the secondary references relate their respective cellular manipulations regarding bo-type oxidase with any indication or hint that such a manipulation will increase, or even effect, production of L-amino acids by the cell. As there is no logical reason to combine these various teachings with the primary teaching of Kojima, the claimed invention cannot be obvious over these references.

## IX. Conclusion

For at least the foregoing reasons, Appellant respectfully submits that the subject matters of Claims 1, 6, and 12-17, each taken as a whole, are patentable. Accordingly, Appellant respectfully requests reversal of the rejections of Claims 1, 6, and 12-17 under section 103(a).

Respectfully submitted,

By: /Shelly Guest Cermak/
Shelly Guest Cermak
Registration No. 39,571

**U.S. P.T.O. Customer Number 38108**
Cermak Nakajima LLP
127 S. Peyton Street
Alexandria, VA 22314
703.717.9387 (v)
703.717.9392 (f)

Date: January 5, 2011

## APPENDIX A: CLAIMS ON APPEAL

1. A method for producing an L-amino acid, comprising:

A) culturing a bacterium belonging to the genus *Escherichia* or a coryneform bacterium in a medium; and

B) collecting said L-amino acid from said medium,

wherein the bacterium has an ability to produce and accumulate the L-amino acid in the medium and has been modified so to have enhanced activity of cytochrome bo-type oxidase by a method selected from the group consisting of

i) increasing the copy number of a gene coding for said oxidase,

ii) modifying an expression regulatory sequence of said gene, and

iii) combinations thereof.

6. The method according to Claim 1, wherein said bacterium has been further modified to be deficient in NDH-II activity by disruption of a gene coding for said NDH-II.

12. The method according to claim 1, wherein said L-amino acid is L-lysine.

13. The method according to claim 1, wherein said L-amino acid is L-threonine.

14. The method according to claim 1, wherein said L-amino acid is L-phenylalanine.

15. The method according to claim 1, wherein said cytochrome bo type oxidase is encoded by cyo operon.

16. The method according to claim 1, wherein said bacterium is *Escherichia coli*.

17. The method according to claim 1, wherein said bacterium is *Corynebacterium glutamicum*.

## APPENDIX B: EVIDENCE

No additional evidence is cited in this Brief.

## APPENDIX C: RELATED PROCEEDINGS

Rejections of claims in this application (09/897,988) were previously appealed to the Board of Patent Appeals and Interferences, Appeal No. 2009-4917, which were reversed in a Decision dated August 11, 2009. A copy of the Decision appears on the following pages.